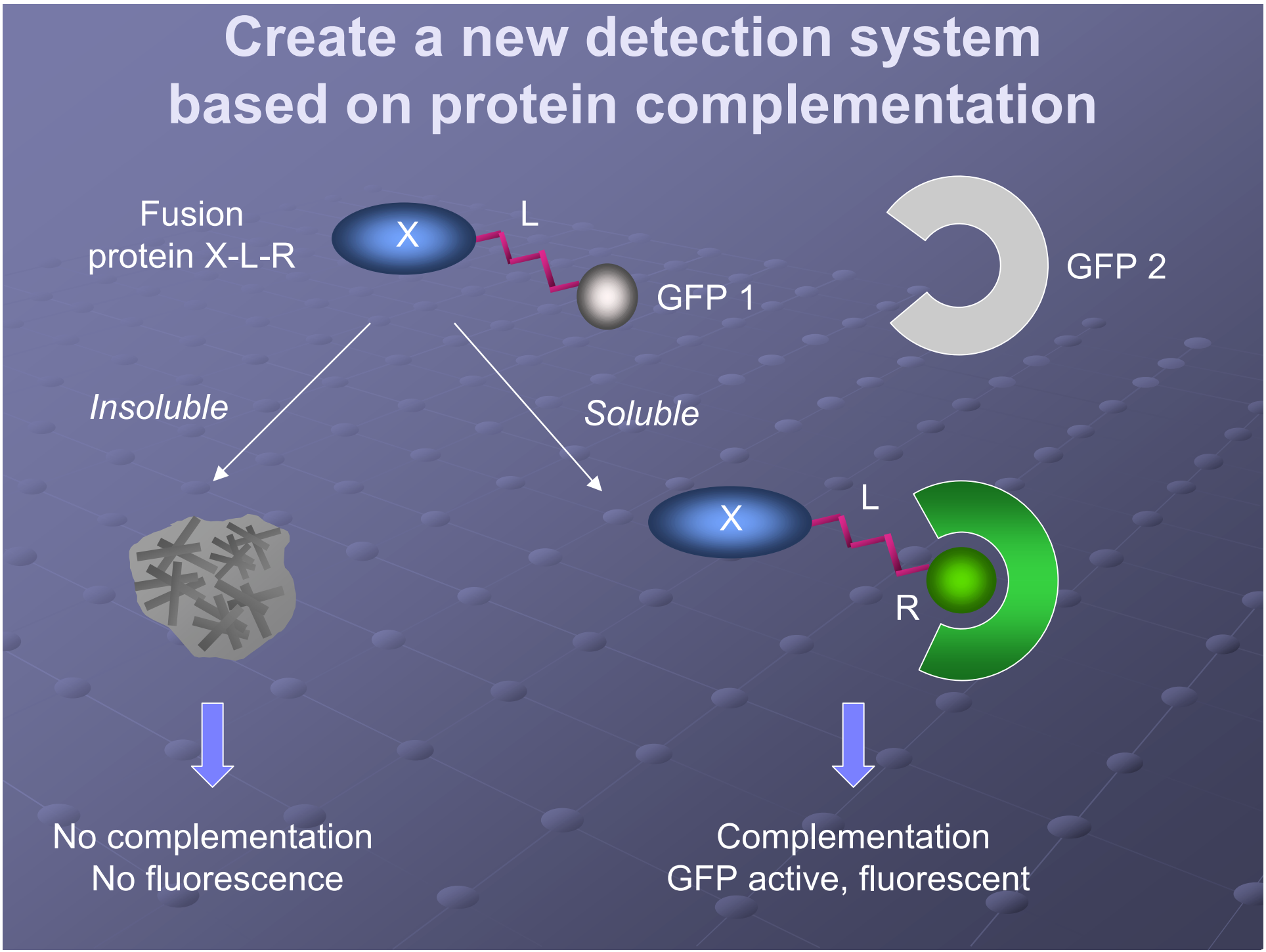
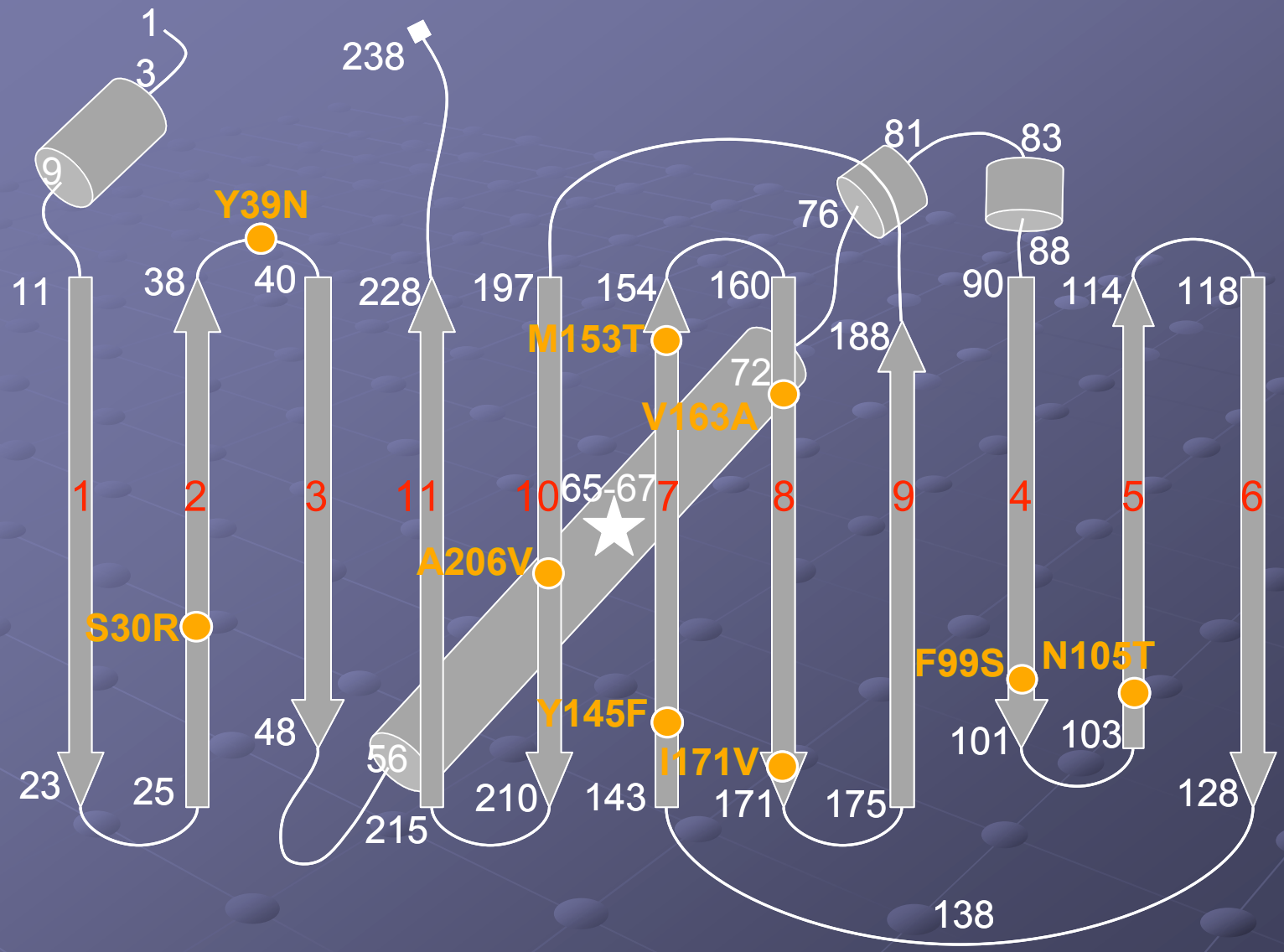
The split-GFP system

Create a new detection system based on protein complementation

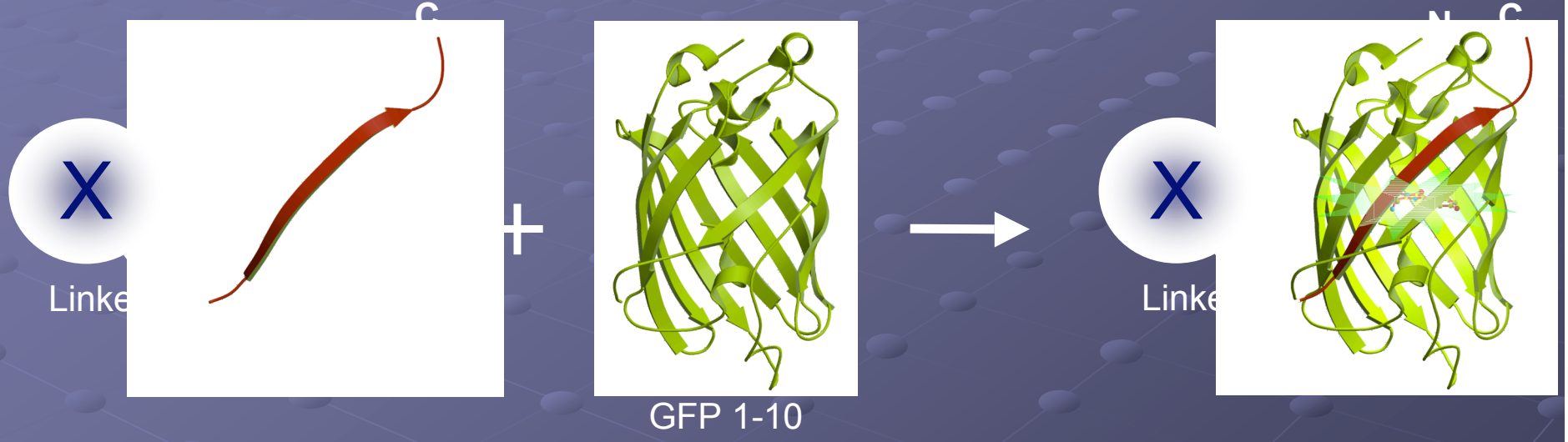


Finding complementing pairs of GFP



Only (GFP 10-11 + GFP 1-9) and (GFP 11 + GFP 1-10) pairs from Superfolder GFP showed complementation and fluorescence.

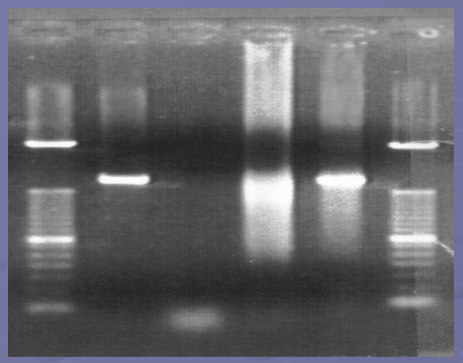
The Split-GFP tagging system



Cabantous S. et al. (2005). "Protein tagging and detection using engineered self-assembling fragments of green fluorescent protein". Nat. Biotechnology 23(1), 102-7.

Improving GFP 1-10 solubility

1



2

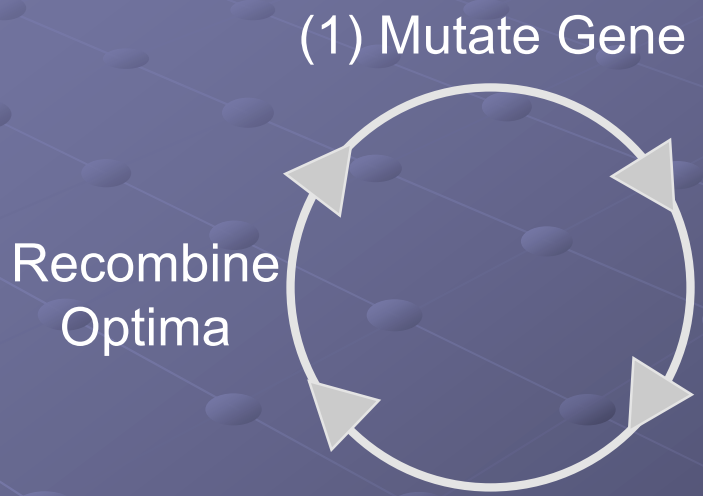
GFP 1-10 LIB
(pET *T7*)

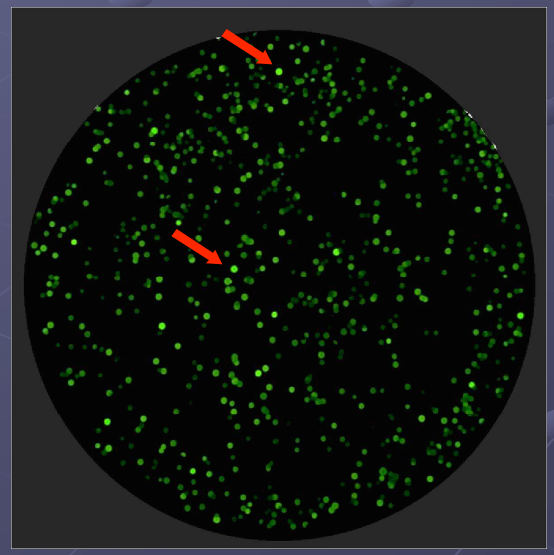
Expression strain
SR-GFP 11 (*tet*)

3

Induction

IPTG,
Tetracycline

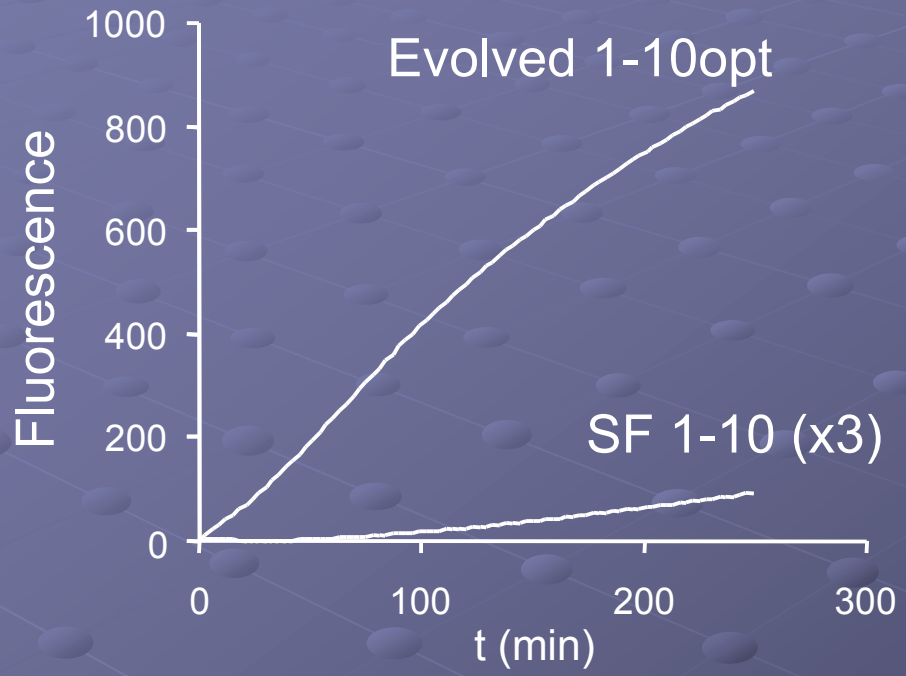




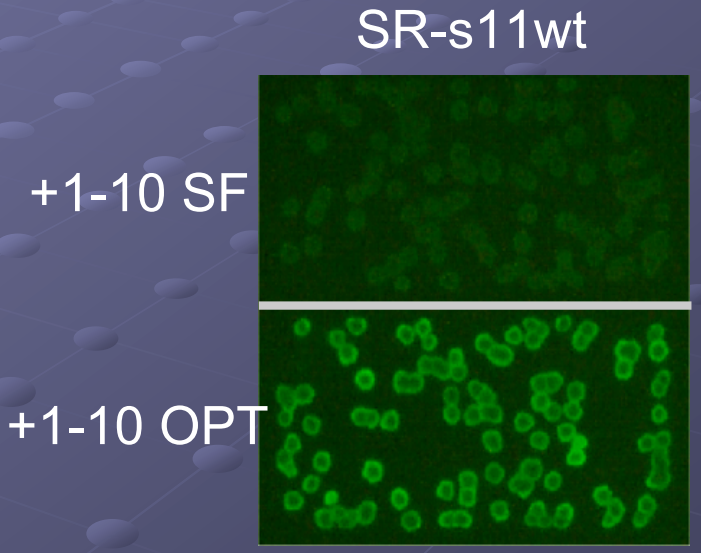
***In vivo* complementation of the two GFP fragments**

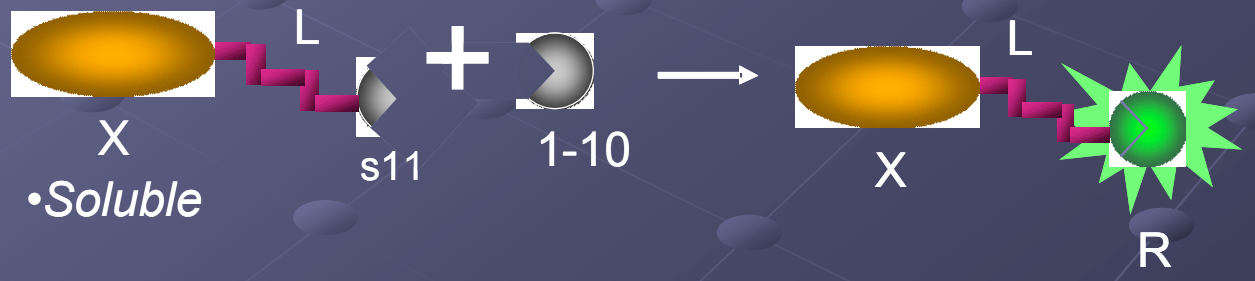
The large fragment 1-10 was engineered to improve performance and solubility

• *In vitro*



• *In vivo*





Small GFP 11 tag was engineered to minimize its effect on passenger folding and solubility

In vitro complementation

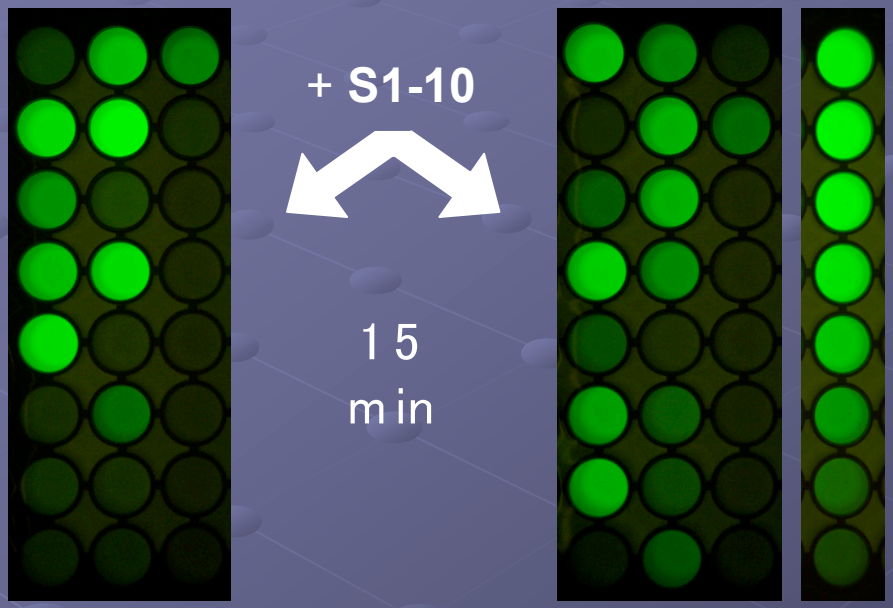
Express X-S11

Fractionate & Centrifuge

soluble

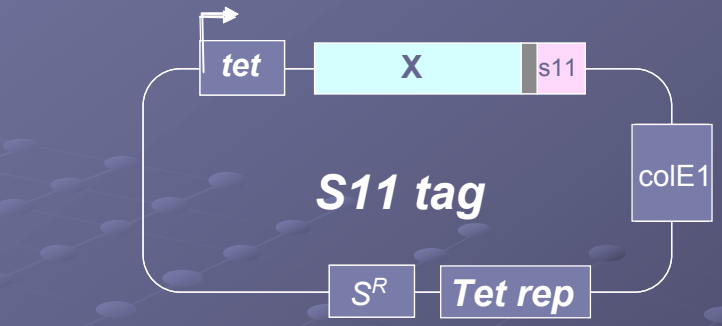
+ 9M Urea

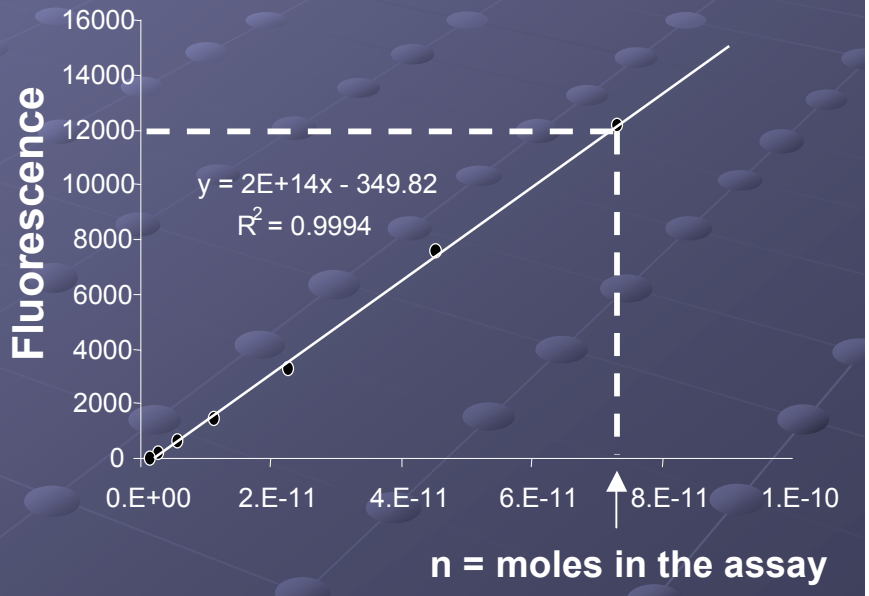
insoluble ©



+ **S1-10**

15 min

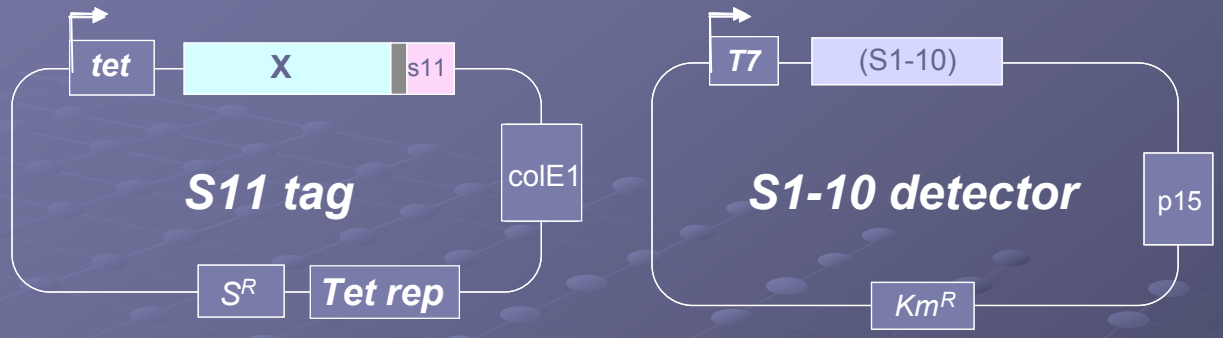


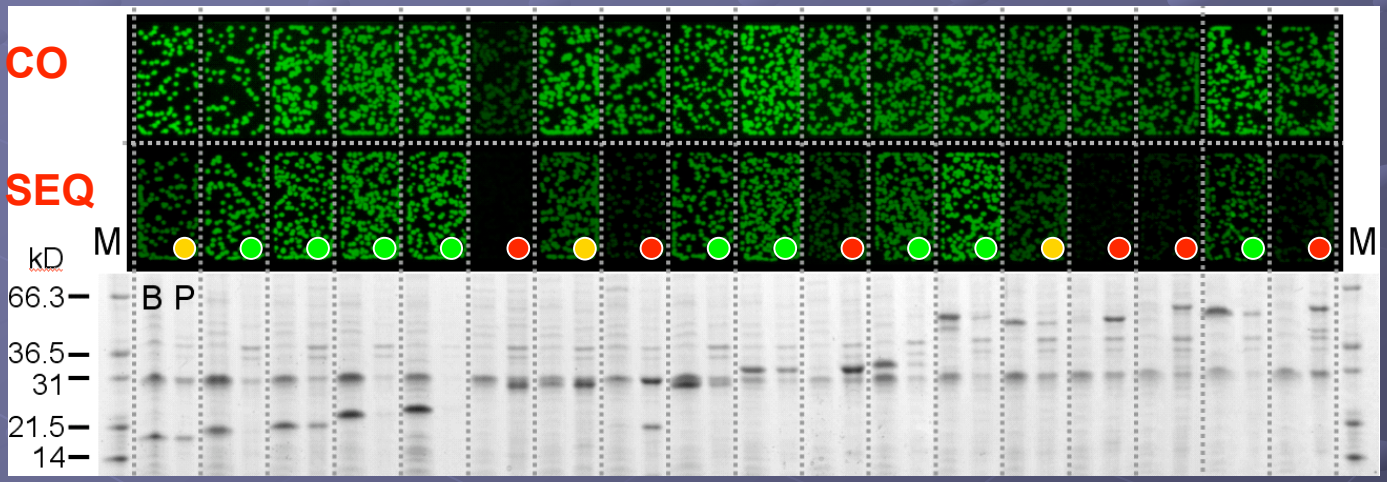


A calibration © with a standard protein enables the quantification of soluble protein from assay fluorescence

In vivo complementation

2 independently-inducible plasmids in *E. coli*





Legend:
- = soluble
- = partially soluble
- = insoluble

Co-induction of tagged protein & detection fragment → *total expression*

Sequential induction of tagged protein then detection fragment → *soluble*

Cabantous et al., Nat. Biotechnology 2005

Applications of Split-GFP

- High throughput Expression Screening: soluble, insoluble, total expression
- Assay of proteins during workup & purification
- Refolding assays: find optimal refolding conditions
- Find soluble mutants of a protein (directed evolution)
- Find soluble domains of a protein